

RootDetection - Reference



www.labutils.de

Citation:

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1 Introduction

RootDetection is an automated tool for evaluating photographs of plant roots.

It detects single strand roots, traces their paths and measures the resulting lengths - completely automatic.

All results are written to an embedded database (SQLite) and can be exported as MS Excel compatible CSV files.

RootDetection also provides tools for manually tracing features like hypocotyls or side roots.

2 Features

Use Cases

- Large scale phenotyping (e.g. for QTL analysis) of roots or hypocotyls

General Features

- Platform independent (Windows, Mac OS X, Linux)
- Comfortable user interface
- Integrated documentation
- Field-tested
- Fast (usually less than 10s per image)
- Free

Automatic Operation

- Batch mode - traces multiple files in a single run
- Label scanner - automatically recognizes QR codes for labelling
- Start line detection - automatically finds a start line marker
- Start point detection - automatically finds root crossing the start line
- Root tracing - automatically traces root path
- End point detection - automatically finds root end

Convenience Features

- Integrated post processing - fix tracing problems, relabel results
- CSV export
- Label generator - create labelling QR codes

3 Requirements

3.1 Software requirements

Java runtime version 6 or later is required. Can be obtained via www.java.com/en/download/manual.jsp.

3.2 Hardware requirements

- 2 GB RAM (4 GB RAM recommended)
- 1024 by 768 pixels

4 Installation

Download **RootDetection** ZIP-archive via www.labutils.de.

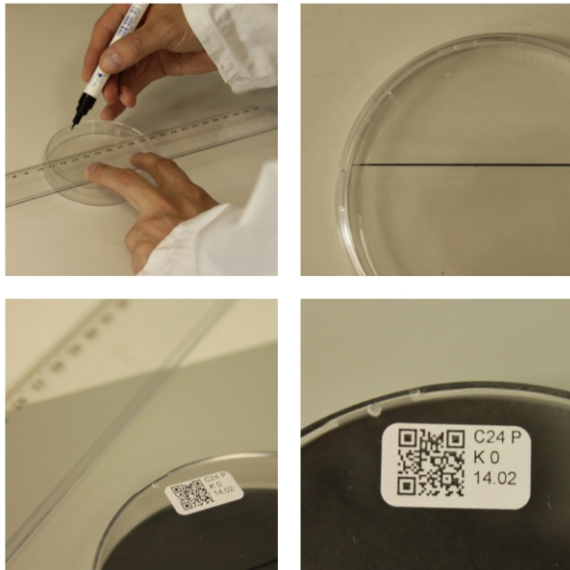
Unpack the archive into your favorite installation location.

The package contains executables for all major platforms:

- rootdetection-xxx.app (MAC OS X)
- rootdetection-xxx.exe (Windows)
- rootdetection-xxx.sh (Linux)

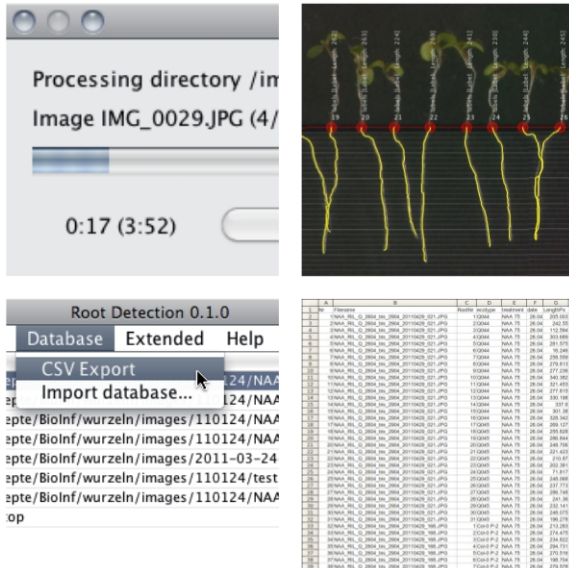
5 Exemplary workflow with RootDetection

1. Preparation



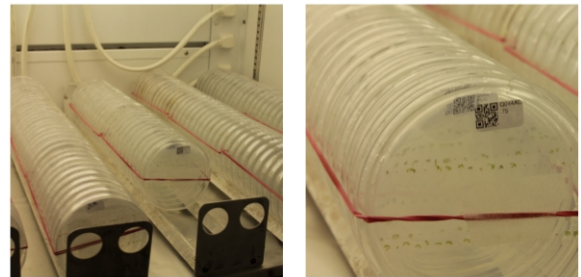
- mark petri dishes with measure line
- apply barcode labels

4. Perform RootDetection



- run fully automated batch mode
- perform optional image post processing
- export data to MS Excel or access data with R package

2. Perform experiment



- proceed with experimental setup

3. Take pictures



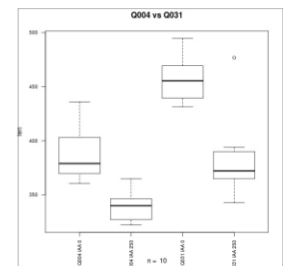
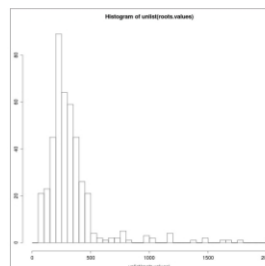
- take pictures of petri dishes with digital camera

5. Perform data analysis via R package



```
> source("../Desktop/RootDetection_Script/RootDetection.R")
> RD_load("../Desktop/results.db")
[[1]]
<SQLiteConnection: DBI CON (2917, 1)

> dir<-RD.list.directories()
> img<-RD.list.images(dir[3])
> roots<-RD.list.roots.max(dir[3])
> roots<-RD.list.roots.max(dir[3],n)
> str(img)
chr [1:44, 1] "/Users/ole/Konzepte/...
- attr(*, "dimnames")=List of 2
..$ : chr [1:44] "1" "2" "3" "4"
..$ : chr "path"
```



- connect to database with R package
- analyse and visualise data (histogram, boxplot,...)

(For more information please refer to the RootDetection R package manual).

6 Image requirements

6.1 Taking pictures / Requirements and Tips

RootDetection can handle any picture in JPEG format.

You can obtain suitable images from:

- Digital single lens reflex camera (DSLR)
- Digital camera
- Scanner

6.2 Requirements

Take a look at the example picture included in the ZIP-archive for proper image settings

You can also download the example picture set from www.labutils.de.

Things to remember:

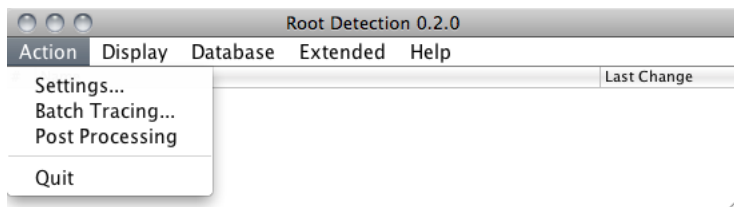
- aim for high contrast between root and background
- adjust light settings to ensure legibility of QR-code
- mark petri dishes with distinct measure lines, preferably by using a template (to ensure more or less consistent positioning of measure lines)

More tips:

- use repro-stand with dark background and diffuse lighting
- avoid reflections on the picture
- avoid direct light on the picture
- increase depth of sharpness (F/11 and higher)
- include scale on every picture (or retain same focal length and image distance for all images)

7 Menu Overview

7.1 Action Menu



7.1.1 Action > Settings...

The 'Settings...' entry of the 'Action' menu will open a file selection dialog and allows you to choose one exemplary image in your picture folder for required image settings.

7.1.2 Action > Batch Tracing...

The entry 'Batch Tracing' opens a folder select dialog and allows you to choose the **picture folder** for a new batch tracing run.

Batch Tracing will use the settings specified in the config.properties file in that folder and will scan and measure roots automatically.

It will create a new '**output**' folder within the **picture folder** to store reference images with the found roots in jpeg format.

7.1.3 Action > Post Processing

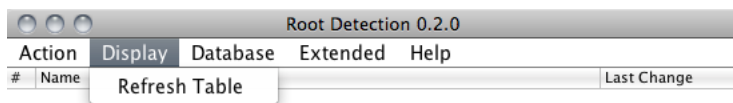
Choose your **picture folder** from the **RootDetection table** and start post processing to fix tracing problems or to relabel results.

When selecting this entry, the currently highlighted folder in the overview table will be opened for post processing. In other words - you cannot post process images or folders, that have not been batch traced before. If you want (or have) to avoid automatic batch tracing before post processing, you can use the 'Manual Batch Trace' mode in the 'Extended' menu.

7.1.4 Action > Quit

Quits **RootDetection**.

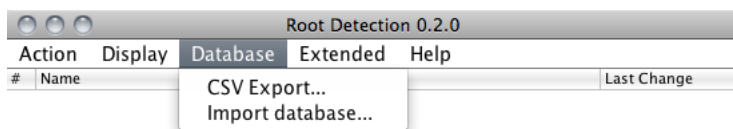
7.2 Display Menu



7.2.1 Display > Refresh Table

Refreshes directory table in program overview after database import or during Batch Tracing.

7.3 Database Menu



7.3.1 Database > CSV Export...

Exports the most recent results of the currently highlighted folder into a CSV file.

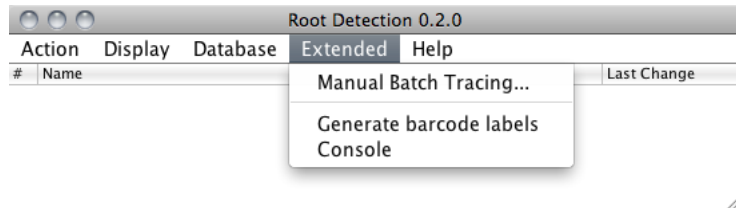
Choose the appropriate '**Files of Type**' option to define the output style of the saved data:

- **'Grouped by Region'** Exports all data grouped by the defined regions. (preferred for Excel import)
- **'List'** Exports all data as a single list. (preferred for automated processing, e.g. with custom software)

7.3.2 Database > Import database

Imports Database from other **RootDetection** installations and from older versions of **RootDetection**.

7.4 Extended Menu



7.4.1 Extended > Manual Batch Tracing...

The manual batch tracing mode is quite similar to the normal post processing mode. The only difference is that it opens and works on all the files in the selected directory, NOT just on the previously scanned images of a batch trace run.

Choose your **picture folder** to start manual processing of your images. This allows you to trace side roots, hypocotyls or other less distinguishable plant features.

7.4.2 Extended > Generate barcode labels

Generates QR-code labels. These labels can be printed on label pads and can be used to automatically assign root detection results to experiment parameters, plant sets and other metadata.

Each line represents **one** label. The label will contain a QR-code encoding the plain text of this line. The QR-code is scaled as large as possible to still fit the label. For increased usability, the plain text is then output into the remaining space. It is split into lines at [Tab] and [Semicolon] characters. This can be used to split the label data into different groups

Example:

```
Mutant1<tab>Treatment1<tab>date
Mutant2<tab>Treatment1<tab>date
```

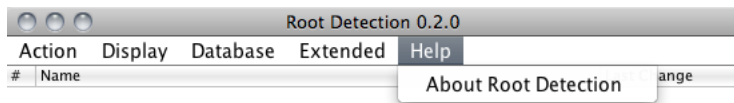
or

```
Mutant1;Treatment1;date
Mutant2;Treatment1;date
```

7.4.3 Extended > Console

Opens the error console. The error console collects error and debugging messages of **RootDetection** and can sometimes contain helpful information when struggling with unexpected problems.

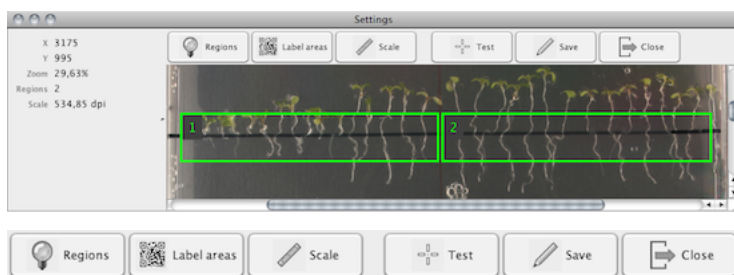
7.5 Help Menu



7.5.1 Help > About RootDetection

Shows the current **RootDetection** version.

8 Settings Window



8.1 Regions

Defines image regions to be scanned. Usually an image contains up to 4 regions with different sets of plants. The definition of the regions allows RootDetection to assign each detected and measured root to be correctly assigned to the right set of plants.

1. Click 'Regions' button
2. Drag mouse for each region
3. Click 'Regions' button again to end region selection

8.2 Label areas

Defines label regions. Each plant region (see above) can have an associated label area where the QR-Code label has been placed. This allows individual labelling of different plant sets within each image.

1. Click 'Label areas' button
2. Drag mouse for each region

8.3 Scale

Defines the image scale. This step is only necessary if you are interested in metric length values (mm). Otherwise all lengths values will be in pixels.

1. Click 'Scale' button
2. Drag mouse

3. Input reference length in mm (no decimal places)

8.4 Test configuration

Applies the current configuration to the image. This quick test can reveal misplaced regions or label areas.

1. Click 'Test' button

8.5 Save

Saves the current settings of image to the config.properties file in the containing folder.

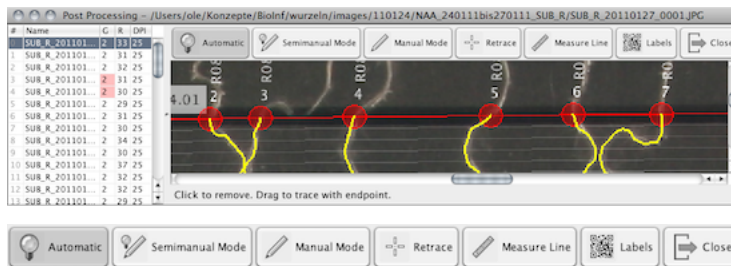
1. Click 'Save' button

8.6 Close

Returns to program overview.

1. Click 'Close' button

9 Post Processing Window



9.1 Keyboard Shortcuts

9.1.1 Viewing/Zooming

- Overview of whole image.
- Original size of image.
- Zoom in.
- Zoom out.
- Turn ON/OFF anti-aliasing in lower magnitude.

9.1.2 Miscellaneous

- **Spacebar** Toogles between 'Automatic', 'Semimanual Mode' and 'Manual Mode'.
- **Ctrl + s** Save image to Database (Windows/ Linux).
- **CMD + s** Save image to Database (MAC OS X).

9.2 Introduction

The tools in the post processing window are context sensitive. Clicking on an existing start point will perform an entirely different action than clicking on an empty image area (removing that root vs. adding a new start point). You can always take a look at the helpful hints given in the status bar of the post processing window.

9.3 Automatic

This mode is the standard post processing mode. It allows for quick touch ups to the scan result (removing obsolete start points, adding missed start points, fixing incorrectly scanned lengths etc.)

- Left click on a start point removes it.
- Left click into an empty area adds a new start point and measures the root.
- Dragging an existing start point traces the root up to the defined end point.

9.4 Semimanual

This mode involves a little more handywork but provides much more flexibility and better results with complicated root paths (lots of overlap, low contrast etc.) You can paint a blue scan region for every root which will be favoured when scanning the root path. This way you can prevent wrong turns, duplicate path traversals etc.

- Left click on a start point removes it
- Left click into an empty area adds a new start point. If you continue to drag the mouse pointer after the initial click, you can define the region in which **RootDetection** will scan for the root. The blue area is the region with the highest weight for the scan.
- Drag any other start point to trace with defined scan region and end point

9.5 Manual Mode

The manual mode behaves exactly like the Semimanual Mode, except that the drawn path is used AS IS for the root. No further scanning or tracing is performed. This mode is intended to be used for very complicated or undistinguishable root paths.

9.6 Retrace

The 'Retrace' function will retrace the complete image. Actually, we hardly ever use this feature at all. But you might find it useful.

A '**Please select**' window will appear, offering three options:

- **Keep start lines** will keep the existing measure lines.
- **Discard start lines** will discard current measure lines and initiate an automatic scan for measure lines.
- **Cancel** will cancel the 'Retrace' function.

9.7 Measure Line

Drag and drop end points of measure line to adjust them.

9.8 Labels

The 'Labels' function will open a window to adjust the label text of each region. This is helpful if the QR-code scanned could not detect the QR-code (or if you haven't used any...).

You can manually input the text for each region

In the window you have three different options:

- **Scan** will rescan the defined label regions.
- **Cancel** will cancel the 'Labels' function.
- **OK** will save your manually entered text changes.

9.9 Close

Returns to program overview.

10 Online video tutorials

For a guided online video tutorial which explains basic functions of **RootDetection** please visit www.labutils.de.

11 License

By using **RootDetection** you need to accept the terms and conditions of the following licenses.

RootDetection is licensed under the MIT License (MIT).

The MIT License (MIT)

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The following libraries were used for the development of **RootDetection**:

Guava r08	Apache License Version 2.0, January 2004, www.apache.org/licenses/
iText 2.0.1	iText is published under 2 different licenses: MPL and LGPL
JGoodies Forms 1.2.1	BSD License
SQLite4java 0.201	Apache License Version 2.0, January 2004, www.apache.org/licenses/
ZXing 1.6	Apache License Version 2.0, January 2004, www.apache.org/licenses/

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